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Determination of *Michaelis-Menten* kinetics for the removal of cyanide by plants

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Abstract

Uptake and toxicity of cyanide were measured with the willow toxicity test in hydroponic solution and in sand; metabolism was measured with a closed-bottle test.
The results were used to evaluate a model that calculates accumulation of cyanide inside plants.
Willow roots and leaves were able to metabolize 7.8 and 10.0 mg CN/(kg plant · h), respectively.
Accumulation of cyanide was seen when the plants were exposed to concentrations > 30 mg CN/L. The plants can according to model calculations survive internal concentrations of 2 mg CN/kg.

Introduction

Vascular plants possess the enzyme β-cyanoalanine synthase which makes them capable of incorporating free cyanide (CN) into the amino acid asparagine. Removal of cyanide by plants is relevant for evaluation of phytoremediation of cyanide polluted soils and water. The present work had three primary objectives. The first was to measure the phytotoxicity of cyanide to willow trees (*Salix viminalis*). The second was to determine *Michaelis-Menten* kinetics for removal of cyanide by willow trees. This provided data for the third objective, a model that describes uptake and degradation of cyanide.

Materials and methods

Toxicity test

Willow cuttings are grown in hydroponic solution or sand and irrigated with cyanide solution.
Transpiration is used as parameter to evaluate toxicity.

Figure 1. Toxicity test with willow trees grown in sand.

Closed-bottle test

Detached leaves or roots or thin slices of stem are placed in a cyanide solution. The concentration of cyanide in the solution is monitored over time.

Figure 2. Closed-bottle test with roots, stem and leaves.

Modelling

Experimental values of v_{max} and K_M are used to calculate concentrations of cyanide inside the different plant compartments.

$$\frac{dm_{root}}{dt} = \text{Uptake} - \text{degradation} - \text{translocation}$$

$$\Downarrow$$

$$\frac{dm_{root}}{dt} = C_{solution} \cdot \text{transpiration} - \frac{v_{max} C_{root}}{K_m + C_{root}} \cdot M_{root} - \text{transpiration} \cdot \frac{C_{root}}{RCF}$$

$$\frac{dm_{stem}}{dt} = \text{Translocation} - \text{degradation} - \text{stemout}$$

$$\Downarrow$$

$$\frac{dm_{stem}}{dt} = \text{Transpiration} \cdot \frac{C_{root}}{RCF} - \frac{v_{max} C_{stem}}{K_m + C_{stem}} \cdot M_{stem} - \text{transpiration} \cdot \frac{C_{stem}}{K_{stem}}$$

$$\frac{dm_{leaf}}{dt} = \text{Stemout} - \text{degradation} + \text{uptake from air} - \text{loss to air}$$

$$\Downarrow$$

$$\frac{dm_{leaf}}{dt} = \text{Transpiration} \cdot \frac{C_{stem}}{K_{stem}} - \frac{v_{max} C_{leaf}}{K_m + C_{leaf}} \cdot M_{leaf} + C_{air} \cdot g \cdot A_{leaf} - C_{leaf} \cdot g \cdot A_{leaf} \cdot \left(\frac{K_{LA}}{\rho}\right)^{-1}$$

Figure 3. Equations used for the modelling.

Results

Toxicity test

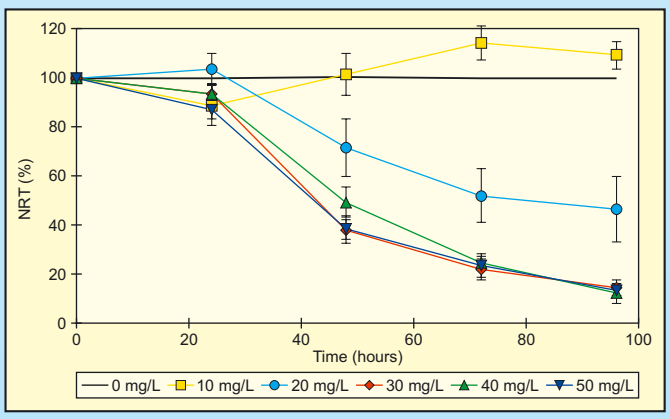

Figure 4. Normalized relative transpiration (NRT) for willow trees exposed to cyanide.

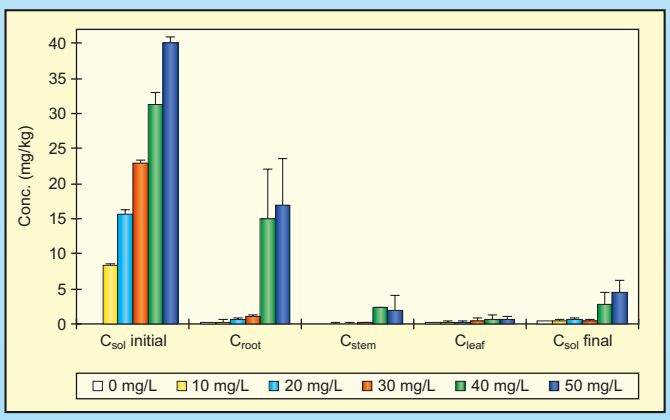

Figure 5. Concentrations in the different compartments after exposure to cyanide.

Closed-bottle test

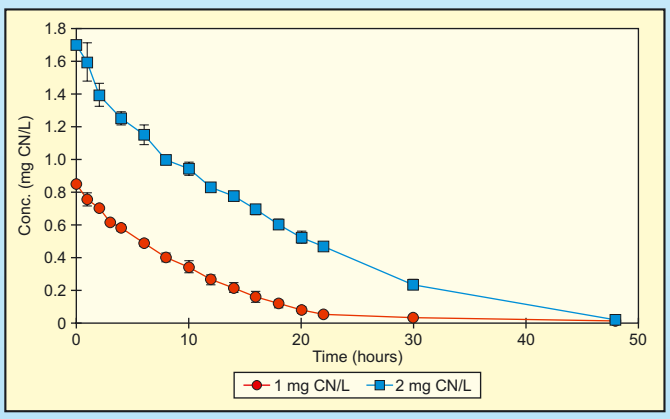

Figure 6. Decrease in concentration when 2 g of leaves are placed in a 200 mL solution containing cyanide.

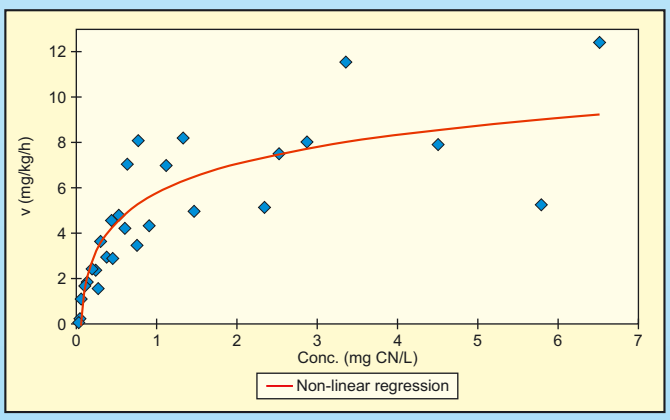

Figure 7. Removal rate of cyanide as a function of substrate concentration for leaves.

Modelling

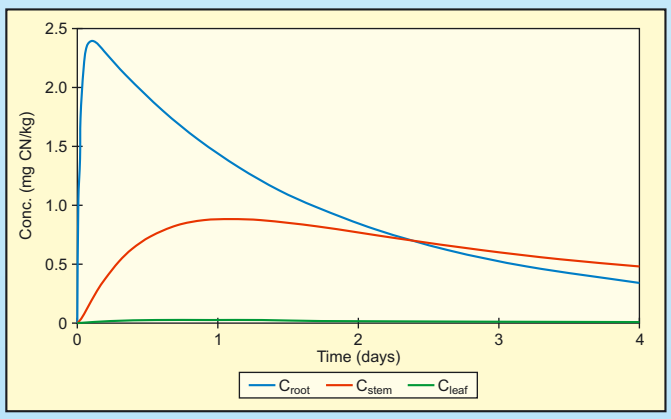

Figure 8. Model calculations of internal concentrations of cyanide when grown in sand and irrigated with 20 mg CN/L solution.

	$C_{solution}$ (mg/L)	C_{root} (mg/kg)	C_{stem} (mg/kg)	C_{leaf} (mg/kg)
Experimental	10	0.23	0.06	0.26
Model	10	0.18	9.0E-05	1.4E-06
Experimental	20	0.72	0.07	0.24
Model	20	0.76	5.2E-04	1.0E-05
Experimental	30	1.09	0.12	0.51
Model	30	1.24	9.2E-04	2.6E-05
Experimental	40	15.00	2.26	0.63
Model	40	16.12	1.28	4.3E-02
Experimental	50	17.03	1.87	6.2E-01
Model	50	17.76	2.59	1.0E-01

Table 1. Comparison between experimental determined and modelled internal concentrations of cyanide.

Conclusion

Toxicity test

- Concentrations > 2 mg CN/l when grown in aqueous solution and > 20 mg CN/l when grown in sand were toxic for the plants.
- When grown in sand, accumulation of cyanide inside the tree was only seen at concentrations > 30 mg CN/L. At this point the trees died.

Closed-bottle test

- *Michaelis-Menten* parameters were determined by non-linear regression:

	v_{max} (mg/kg/h)	K_M (mg/L)
Roots	6.86 ± 0.86	0.44 ± 0.16
Stem	1.62 ± 0.33	0.01 ± 0.20
Leaves	9.55 ± 0.52	0.52 ± 0.10

Table 2. Experimental determined values of v_{max} and K_M.

Modelling

- The model is only valid when no toxic effects are observed. Otherwise the degradation rate is affected by the toxicity.
- Plants can according to model calculations survive internal concentrations of ~2 mg CN/kg.
- Accumulation of cyanide inside the plants was only found when uptake > degradation.